UNIVERSITI PUTRA MALAYSIA

THE EFFECTS OF VARYING DIETARY PROTEIN LEVELS ON GROWTH PERFORMANCE OF THE MEKONG RIVER CATFISH PANGASIUS HYPOPHTHALMUS FRIES.

HENG NGAN

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By

HENG NGAN

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Institute of Bioscience
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DEDICATION

To the loving and sacred memory of my parents

Mr. and Mrs. HOR NGOURN

Who left me forever during

The Republic State 1972 period of Cambodia

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Office Analytical Chemist
APHA	American Public Health Association
AVG	Average
BFSPRC	Bati Fish Seed Productions and Research Center
C	Clarias
EFA	Essential Fatty Acid
FCR	Feed conversion ratio
FG	Fat Gain
GE	Gross Energy
GR	Growth Rate
Kcal	Kilocalorie
Lgh	Length
Mg/Kcal	Milligrams per Kilocalorie
NRC	National Research Council
NFE	Nitrogen Free Extract
PADEK	Partnership Development for Kampuchea.
P:E	Protein to Energy ratio
PER	Protein Efficiency Ratio
PG	Protein Gain
pH	Potential of Hydrogen ion

Rpct	Replicate
SD	Standard division
SGR	Specific Growth Rate
NPU	Net Protein Utilisation
Wt	Weight

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

THE EFFECTS OF VARYING DIETARY PROTEIN LEVELS ON THE GROWTH PERFORMANCE OF THE MEKONG RIVER CATFISH *PANGASIUS HYPOPHTHALMUS* FRIES

By

HENG NGAN

April, 1999

Chairman: Che Roos Saad, Ph.D.

Faculty: Institute of Bioscience

Seven practical diets were formulated to evaluate the growth, survival rate, body composition and nutrient gain of the Mekong River catfish *Pangasius hypophthalmus* fries under different dietary protein levels and protein to energy ratios. The diets contained 15.76, 20.08, 24.36, 28.69, 33.01, 37.33 and 41.63% protein and protein energy (P:E) ratio of 58.0, 70.7, 82.0, 94.0, 105.0, 114.0 and 123.0 mg/Kcal respectively. The experimental fish ranged 2.64 g - 2.75 g were nursed in twenty one 1 m^3 hapas which were installed in a 600 m^2 earthen pond at a stocking density of 15 fishes/hapa. All treatments were assigned at random and triplicated. Fish were fed at 10% of the total body weight daily for 90 days. Fifty percent of fish were sampled every fortnight for total length and weight.

However, on termination of the experiment, individual fish weight and length were recorded.

Fishes fed with diets containing 33.01, 37.33 and 41.63% protein showed significantly higher growth ($p<0.05$) than fish receiving diets containing 15.76, 20.08, 24.36 and 28.69% protein. The highest growth was recorded in fish fed with diet containing protein 37.33% and P:E 114mg/Kcal while the lowest growth was observed in fish fed with diet containing 15.76% protein. However, fish fed with diets containing 41.63% and 123 mg/Kcal did not perform as well as fish fed with diet containing 37.33% protein.

The fish growth rate increased significantly ($p<0.05$) with increasing dietary protein levels up to 37.33%. The growth rate decreased as the dietary protein was increased beyond 37.33% protein level.

There was significantly positive correlation between specific growth rate (% per day) and protein to energy (P:E) ratio with an equation of $Y= 0.6512 + 0.0055 X$, $r = 0.9533$.

In conclusion, formulated diet containing 37.33% protein level, 3270 Kcal/Kg of energy and P:E ratio of 114 mg/Kcal favour maximum growth (2413%), highest survival rate (100%), high protein gain (2576%), and feed conversion ratio (3.64) for *Pangasius hypophthalmus* fries.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat keperluan untuk ijazah Master Sains

KESAN PERBEZAAN KANDUNGAN PROTEIN KE ATAS PERTUMBUHAN FRI IKAN PATIN MEKONG PANGASIUS HYPOPHTHALMUS

Oleh

HENG NGAN

April, 1999

Pengerusi: **Che Roos Saad, Ph.D.**

Fakulti: **Institut Biosains**

Tujuh diet telah dirumuskan dan diuji untuk menilai pertumbuhan, kadar hidup, komposisi badan dan pertambahan nutrien anak ikan patin Sungai Mekong *Pangasius hypophthalmus*. Diet-diet mengandungi 15.76, 20.08, 24.36, 28.69, 33.01, 37.33, dan 41.63% protein dan nisbah protein kepada tenaga (P:E) 58.0, 70.7, 82.0, 94.0, 105.0, 114.0, dan 123.0 mg/Kcal masing-masing. Ikan kajian (2.64 g - 2.75 g/ekor) dimasukkan ke dalam dua puluh satu buah $1m^3$ hapa yang diletakkan dalam sebuah kolam tanah berukuran 600 m^2 dengan kadar kepadatan 15 ekor anak ikan/hapa. Kesemua rawatan dilakukan secara rawak dengan 3 replikasi. Anak ikan diberikan makanan rumusan sebanyak 10% jumlah berat badan setiap hari selama 90 hari. Lima puluh peratus ikan disampel setiap dua minggu untuk ukuran jumlah panjang dan berat. Di akhir ujian, setiap individu ikan diukur untuk berat dan panjang.

Ikan yang diberi makan diet mengandungi 33.01, 37.33 dan 41.63% protein telah menunjukkan pertumbuhan yang lebih tinggi ($p<0.05$) daripada ikan yang menerima diet mengandungi 15.76, 20.08, 24.36 dan 28.69% protein. Pertumbuhan yang paling tinggi telah dihasilkan oleh ikan yang memakan diet mengandungi protein 37.33% dan tenaga 114 mg/kcal sementara pertumbuhan yang paling rendah telah didapati dari ikan yang memakan diet yang mengandungi 15.76% protein. Bagaimanapun ikan yang diberi makan diet mengandungi 41.63% dan tenaga 123 mg/kcal tidak menunjukkan pertumbuhan yang lebih baik dari ikan yang memakan diet mengandungi 37.33% protein.

Kadar pertumbuhan ikan meningkat dengan bererti ($p<0.05$) dengan peningkatan protein dalam diet sehingga 37.33%. Bagaimanapun pertambahan protein dalam makanan melebihi 37.33% memberi pertumbuhan yang menurun kepada ikan.

Terdapat korelasi yang positif antara kadar pertumbuhan spesifik (%/hari) dan nisbah protein kepada tenaga dan regressi yang didapati ialah $Y = 0.6512 + 0.0055 X$, $r = 0.9533$.

Sebagai kesimpulan, rumusan diet yang mengandungi 37.33% protein, 3270 kcal/kg tenaga dan nisbah protein kepada tenaga 114 mg/kcal merangsangkan pertumbuhan yang maksimum (2413%), kadar hidup yang tinggi (100%), pertambahan protein yang tinggi (2576%) dan nisbah pertukaran makanan yang terbaik (3.64) bagi anak ikan *Pangasius hypophthalmus*.

CHAPTER I

INTRODUCTION

Background of the Study

The Mekong River catfish *Pangasius hypopthalmus* is one of high value fish species, which is commonly cultured in small and large scale and is well accepted by Cambodians population. This species has been largely cultured in ponds, cages and pens since long time in Thailand, Vietnam and Laos (Hora and Pillay, 1962; Ling et al., 1965; Ling, 1966). It has been classified as a carnivore in open water but has an omnivorous behaviour when maintained in captivity (Jhingran and Gopalakrishnan, 1974).

Its seed supply from the wild is not only inadequate but has also declined. In addition, fish price is relatively high while attempts to artificially breed the species have yet to succeed. All these factors are limiting to the aquaculture production of *P. hypopthalmus*. The catfishes have an aquacultural importance because of their high growth rate, disease resistance and amenability to high density culture which is related to their air breathing habits (Huisman and Ritcher, 1987; Haylor, 1993). In culturing fish in captivity, nothing is more important than well balanced diets and adequate feeding. If there is no utilisable feed intake by the fish, then there will be no growth and death eventually is the final result. An undernourished or malnourished fish is never able to maintain its health and be productive, regardless of the quality of its environment.

Statement of the Problem

P. hypophthalmus in particular is a valuable cultured specie and is gradually losing its importance in the culture sector due to a number of constraints. Prominent among them is the lack of research to improve the feed quality and the culture technology that is adopted by new entrants to the industry without risking any investment. Generally, fish farmers have been utilising biological waste products, which make poor quality feed diet (Nuov and Nandeesha, 1992). Sometimes during freshwater fishing season, farmers may feed their fish with available trash fish. Besides lacking in feed materials these farmers also lack in feeding strategy. They sometimes overfeed their fish, which not only pollutes their ponds but also, causes wastage of protein source.

Hogendoorn (1980) concluded that the inadequate performance of fry on artificial diet is caused by poor utilisation. Learning to accept artificial diet appears to be the dominant factor in food selectivity. However, it is important to use feeds with suitable dimension and texture to optimise consumption and help maintain good water quality (Knights, 1983). Feeding of *P. hypophthalmus* fries is believed to be strongly influenced by food quality in relation to the weight and size of the fish fry. However, the ingredients should be evaluated prior to formulating the diets in order to determine the optimum dietary protein level by utilising the local ingredients, which are available in the country.

The need for suitable artificial diet for *P. hypophthalmus* fries to totally replace on-farm feed is very essential. This will ensure adequate amount supply of fingerlings and consequently will increase the production of marketable size fish. It is proposed that this study will find out a way towards development of an appropriate artificial diet for *P. hypophthalmus*.fries. Diets formulated will use the local ingredients, which are available in the country. However, the ingredients will be evaluated first priors to formulating the diets in order to determine the optimum dietary protein level. It is hoped that the details regarding feed size, form, feed ingredients, feed composition and protein levels in the artificial feeds given will be defined later.

Objective of the Study

To study the effect of variable dietary protein levels on survival and growth rate of *P. hypophthalmus* fries by utilising local feed ingredients.

CHAPTER II

LITERATURE REVIEW

Nutrient Requirements

The nutrient requirements of fish (finfish and crustaceans) for growth, production, and order normal physiological functions are similar to those of land animals. They need protein, minerals, vitamins and growth factors, and energy sources. The nutrients may come from natural aquatic organisms or from prepared feeds. If fish are held in an artificial confinement where natural food are absent, such as raceways, their feed must be nutritionally complete. However, where natural food is available and supplemental feeds are fed for additional growth, the feeds may not need to contain all of the essential nutrients (Lovell, 1989).

Notable nutritional differences between fishes and farm animals are as follows: (a) energy requirements are lower for fish than for warm-blooded animal, thus giving fish a higher dietary protein to energy ratio; (b) fish require some lipids that warm-blooded animals do not, such as omega-3 (n-3) series fatty acids for some species and sterols for crustaceans; (c) the ability of fish to absorb soluble minerals from the water minimises the dietary need for some minerals; and (d) fish have limited ability to synthesise ascorbic acid and must depend upon dietary sources (Lovell, 1989).

Nutritional requirements of fish do not vary greatly among species. There are exceptions, such as differences in essential fatty acids, requirement for sterols, and ability to assimilate carbohydrate, but these often can be identified by warmwater or coldwater, finfish or crustaceans, and marine or freshwater species. The quantitative nutrient requirements that have been derived for several species have served adequately as a basis for estimating the nutrient needs of others. As more information becomes available on nutrient requirements of various species, the recommended nutrient allowances of diets for specific needs of individual species become more refined (Lovell, 1989).

Optimal Dietary Protein Requirement

Young catfish requires a higher level of protein than larger fish. Mangalik (1986) showed that 3 g channel catfish require almost 4 times more protein per day than 250 g fish for maximum growth, but the ratio of protein to energy in the diet did not change much.

Protein is the major organic component in fish tissue, making up roughly 65-75% of its total dry weight. Protein is usually given more attention in any diet formulation as it represents the major and most expensive component of feeds

(Santiago and Reyes, 1991; Murai, 1992; Van Der Meer et al., 1995; Catacutan and Coloso, 1995). Fish utilizes protein to obtain amino acids that are absorbed into the blood to the organ tissues through the intestinal tract (Wilson, 1989). Hence, information regarding protein requirement is essential in the formulation of well-balanced low cost artificial diets (Strottup et al., 1986).

Santiago and Reyes (1991) noted that the young of several warmwater fish such as Nile tilapia and bighead carp manifest growth depressions in response to excessive levels of protein when isocaloric diets were tested. Wilson (1989) suggested that the dietary protein requirement is also affected by the quality of protein found in the test diets. One example is casein, which is known to be lacking in arginine for most fishes.

In fishes, the optimal amount of protein in the diet is important because extremely low or high protein level may result in poor growth and increased susceptibility to disease and parasites. Furthermore, optimal protein content in the diet will reduce the amount of utilized protein. Excess protein makes the diet unnecessarily expensive (Chuapoehuk, 1987; Santiago and Reyes, 1991). However, some of these values appear to have been overestimated because of inadequate information on one or more of the following dietary factors: a) energy concentration of the diet; b) digestibility of the dietary protein and; c) the amino acid composition of the protein sources (Wilson, 1989). Several authors have found that the protein requirements of fish generally decrease with increasing size and age. It is, however, unlikely that marked differences would occur between fish species. Differences in amino acid requirements are usually found in the pathways of control or mechanisms involved in amino acid oxidation (Cowey, 1994).

The quality or amino acid composition of protein is the most important factor in optimizing utilization of dietary proteins. Studies on catfish fingerlings have shown that better feed efficiency can be obtained from a well balanced diet containing 24% protein than from a poorly balanced diet containing 36% protein (Andrews, 1977). In most animal feeds, a deficiency in methionine or lysine is corrected by the addition of synthetic free amino acids to the formulation. However, studies have indicated that free methionine and lysine are poorly utilized by catfish and provide little or no benefit to catfish feeds (Andrews, 1977). Thus amino acid balance has to be achieved by using combinations of natural protein sources.

Several studies have indicated that fishmeal is a desirable ingredient in catfish feeds (Andrews, 1977). The growth enhancing factor has been shown to be in the non lipid residue of fishmeal. It has not been ascertained if the growth effect is due to amino acid availability or to unidentified growth factors.

The amount of protein required by catfish depends upon the digestibility and amino acid composition of the protein. The size of fish, temperature and energy level of the diet also may influence protein requirement. When large channel catfish are fed as much as they will eat and the diet is balanced in amino acids and energy, 25 to 30% protein is adequate (Andrews, 1977). When the feeding rate is restricted, as in pond culture, higher protein levels have proven beneficial. Fingerlings respond to higher protein levels of 30 to 36%. Protein conversion tends to be optimal in the lower levels of these two ranges, although total production is greater at the higher levels.

Clarias species have a protein requirement of about 40% for optimal growth (Van Weerd, 1995). Several researchers reported that feed for *C. anguillaris* is estimated to be 40% protein (Madu and Tsumba, 1989); *Clarias batrachus* requires a 37 - 40% protein (Chuapoehuk, 1987; Singh, 1994); *Clarias isheriensis* requires a 37 - 40% protein (Fagbenro, 1992) and *Clarias gariepinus* is recommended to be 40% protein (Gad et al., 1989). In another study of Wiang and Chuapoehuk (1987) mentioned that *C. batrachus* fry are able to produce optimal growth with diet containing 30% protein.

Venkatesh et al. (1985) reported that animal protein component is a better source of protein in the diet of *C. batrachus* for satisfactory growth and that fish meal can be incorporated in the diet with an advantage. Degani et al. (1989) showed that African catfish digests a high animal protein diets more efficiently than a plant protein diet.

Chuapoehuk and Pothisoong (1985) fed *Pangassius sutchi* fry with diets containing 20, 25, 30, 35, 40 and 50% protein in concrete tanks for 60 days. They concluded that, a minimum level of 25% protein is needed in the diet for optimum growth of catfish fry. However, Pathmasothy and Lim (1987) reported that *P. sutchi* fingerling fed with 24% protein diet in pond had an inferior growth than those fed with 32% protein diet, indicating a need for higher crude protein content in their food. Similarly Aizam et al. (1983) found that the *P. sutchi* fingerlings fed with 30% dietary protein levels in glass aquarium showed the best growth compared to diets which contained 20 and 40% protein. In another study, Mollah and Sarder (1991) observed the highest growth rate of *P. pangasius* in ponds when fed with the diet containing 35.95% protein.

Results of earlier studies have indicated large variation in the optimal dietary protein requirements among various fish species (Table 1). These differences were mainly attributed to the variation in the culture techniques, environment conditions and diet compositions (Garling and Wilson, 1976; Shiau and Huang, 1989; Li, 1989; Santiago and Reyes, 1991). Within the same species, the growth response of fish to variable protein feeds is influenced by size of fish, feeding rate and frequency, water temperature, stocking density, protein quality, and availability of natural foods (National Research Council, 1983). The variability is also attributed to protein source used (New, 1976; Tacon and Cowey, 1985). Exact protein requirement will undoubtedly vary with alterations of the amino acid profile, variation in dietary supplementation other than protein and